Litton BIONETICS

MUTAGENIC EVALUATION OF
COMPOUND FDA 71-85
HYDROLYZED VEGETABLE
PROTEIN (SOY)

A28

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LBI PROJECT #2468

MUTAGENIC EVALUATION OF COMPOUND FDA 71-85 HYDROLYZED VEGETABLE PROTEIN (SOY)

SUBMITTED TO

FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND

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Litton BIONETICS

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EVALUATION SUMMARY

Compound FDA 71-85, Hydrolyzed Vegetable Protein (Soy), did not exhibit genetic activity in any of the in vitro assays included in this evaluation.

DATE: 12-18-74

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Mutagenic Evaluation of Compound 71-85

I. OBJECTIVE

The objective of this study was to assess the genetic activity of the test material in microbial assays with and without the addition of mammalian metabolic enzyme preparations.

II. MATERIALS

A. Test Material

Hydrolyzed Vegetable Protein (Soy)
Hercules #700 #1-203 BW

B. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from liver, lung and testes of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and primate - Macaca mulatta adult males.

C. Indicator Organisms

The indicator organisms used for all tests are described below:

- Saccharomyces cerevisiae, strain D4: $\frac{\alpha}{a}$, $\frac{\text{ade 2-2}}{\text{ade 2-1}}$, $\frac{\text{try 5-12}}{\text{try 5-27}}$

- Salmonella typhimurium, strains:

  TA-1535; hisG, uvrB, rfa (missense mutation)
  TA-1537; hisC, uvrB, rfa (- frameshift mutation)
  TA-1538; hisD, uvrB, rfa (+ frameshift mutation)

D. Reaction Mixture

The following reaction mixture was employed in the activation tests:

	Component	Final Concentration/ml
1.	TPN (sodium salt)	6 µM
2.	Isocitric acid	49 µM
3.	Tris buffer, pH 7.4	28 µM
4.	$MgCl_2$	1.7 µM
5.	Isocitric dehydrogenase	1.0 Unit
6.	Tissue homogenate or cell fraction	72 mg

Components 1-4 were combined and frozen as a "core" reaction mixture to which the other components were added just prior to use.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

ASSAY	CHEMICAL[a]	SOLVENT	PROBABLE MUTAGENIC SPECIFICITY[b]
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrosofluorene	Dimethylsulfoxide[c]	FS
	Quinacrine or Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS

[a] Concentrations given in the Results Section.

[b] BPS = base-pair substitution; FS = frameshift.

[c] Previously shown to be non-mutagenic, see Appendix.

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against strains TA-1537 and D4 over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival dose was determined from the survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

Only three bacteria strains were tested in qualitative plate tests. In the non-activation procedure, approximately 10^9 cells of a log phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (Test, Positive Control and Solvent Control) was done in duplicate. The results were scored as + or -. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non-activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^9 cells/ml and 5×10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in 30 ml plastic tissue culture flasks. Cells plus appropriate volume(s) of the test chemical were added to the flasks to give a final volume of 2 ml. Solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the flasks were set in ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non-activation tests except that the cell densities were increased approximately five-fold for working stock suspensions. Measured amounts of the test and

control chemicals plus 0.25 ml of the stock cell suspension were added to a 30 ml plastic tissue homogenate. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non-activation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

1. Mice

Male mice (sufficient to provide the necessary quantities of organs for testes, lung and liver homogenates) were killed by cranial blow, decapitated and bled. The three organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

2. Rats

The same procedures as described for mice were used for this mammal.

3. Primates

The liver, lungs and testes were aseptically removed from freshly killed adult male rhesus (M. mulatta) monkeys. Each organ was cut into a number of pieces each sufficient for one week's studies. The tissues were labeled and frozen at -80°C until needed. Tissue homogenates and 9,000 x g supernatants were prepared as described for mice.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, in bound data books. Information necessary for identification of the specific experiment as well as the presence of any contaminant micro-organisms was recorded with each set of plate counts. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated.

Frequencies were mechanically calculated and double checked. All data presented in the Results Section of this report consists of the actual sum of all raw data plate counts and only the frequencies are calculated figures.

IV. SOLUBILITY PROPERTIES OF THE TEST COMPOUND

1. NAME OR DESCRIPTION OF TEST COMPOUND:
   FDA 71-85
   Hydrolyzed Vegetable Protein (Soy)
   Hercules #700 #1-203 BW
2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY OF THE TEST CHEMICAL UNDER TREATMENT CONDITIONS: Soluble in phosphate buffer pH 7.4. Test chemical was in solution at all dose levels.

3. OTHER COMMENTS: This chemical acted as an enriching agent for the selective minimal medium probably because as a hydrolyzed protein product it contained some histidine.

V. TOXICITY AND DOSAGE DETERMINATIONS COMPOUND FDA 71-85

		D4	TA-1537
	Dose No.	% Concentration	% Concentration
Range of concentrations of the test compound used to determine the 50% survival level	1	1	0.1
	2	2	0.5
	3	3	1.0
	4	4	2.5
	5	5	5.0
	Dose No.	% Survival	% Survival
Survival Results	Control	100	100
	1	100	100
Test Date: _____	2	100	100
	3	100	100
	4	100	100
	5	100	100
	Dose	% Concentration	% Concentration
Concentrations of the test chemical required for mutagenicity tests	Plate Test	-	5.0
	¼ 50% Survival	-	-
	½ 50% Survival	-	-
	Other	H 5.0	H 5.0
		L 2.5	L 2.5

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VI. NON-ACTIVATION PLATE TESTS

DATE: 9-19-74

Test	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	EMS	0.05 ml undiluted chemical	$>10^3$	$>10^3$				
	QM	0.25 mg			56	43		
	NF	0.25 mg					25	31
SC	SALINE	-	3	0	3	4		
	DMSO	<10%					3	5

NOTE: PC = positive control
SC = solvent control
T-1 = trial 1
T-2 = trial 2
EMS = ethyl methanesulfonate
QM = quinacrine mustard
NF = nitrosofluorene
DMSO = dimethyl sulfoxide
(c) = contamination present

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NON-ACTIVATION PLATE TESTS

DATE: 9-19-74

Test	Compound	Concentration	TA-1535		TA-1537		TA-1538	
			T-1	T-2	T-1	T-2	T-1	T-2
TC	FDA 71-85	5.0%	4	1	8	5	11	10

NOTE: TC = test compound
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

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VII. ACTIVATION PLATE TESTS

SPECIES: Mouse

DATE: 9-19-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	$>10^2$	$>10^2$				
		AAF	1.25 mg			$>10^2$	$>10^2$	$>10^2$	$>10^2$
	Lu	DMNA	25 µmoles	7	3				
		AAF	1.25 mg			17	18	45	35
	T	DMNA	25 µmoles	(c)	5				
		AAF	1.25 mg			23	16	16	13
SC	"	DMNA	25 µmoles	3	4				
	-	AAF	1.25 mg			15	19	17	14
	-	Saline	-	3	4				
	-	DMSO	<10%			12	14	15	13

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: Mouse

DATE: 9-19-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
TC	Li	FDA 71-85	5.0%	11	10	14	28	40	56
	Lu	FDA 71-85	5.0%	14	13	26	26	60	49
	T	FDA 71-85	5.0%	18	8	29	28	65	54

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: Rat

DATE: 9-19-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	$>10^2$	$>10^2$				
		AAF	1.25 mg			$>10^2$	$>10^2$	$>10^2$	$>10^2$
	Lu	DMNA	25 µmoles	2	3				
		AAF	1.25 mg			17	8	28	20
	T	DMNA	25 µmoles	3	6				
		AAF	1.25 mg			7	8	14	9
SC	-	DMNA	25 µmoles	3	4				
	-	AAF	1.25 mg			15	19	17	14
	-	Saline	-	3	4				
	-	DMSO	<10%			12	14	15	13

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: Rat

DATE: 9-19-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
TC	Li	FDA 71-85	5.0%	13	10	29	6	52	51
	Lu	FDA 71-85	5.0%	11	11	25	23	50	46
	T	FDA 71-85	5.0%	19	10	21	21	46	46

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2

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ACTIVATION PLATE TESTS

SPECIES: Monkey

DATE: 9-19-74

Test	Organ	Compound	Concentration/plate	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
PC	Li	DMNA	25 µmoles	$>10^2$	$>10^2$				
		AAF	1.25 mg			$>10^2$	$>10^2$	$>10^2$	$>10^2$
	Lu	DMNA	25 µmoles	3	4				
		AAF	1.25 mg			10	8	13	10
	T	DMNA	25 µmoles	3	3				
		AAF	1.25 mg			10	7	11	11
SC	-	DMNA	25 µmoles	3	4				
	-	AAF	1.25 mg			15	19	17	14
	-	Saline	-	3	4				
	-	DMSO	<10%			12	14	15	13

NOTE:
PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: Monkey DATE: 9-19-74

Test	Organ	Compound	Concentration	TA-1535 T-1	TA-1535 T-2	TA-1537 T-1	TA-1537 T-2	TA-1538 T-1	TA-1538 T-2
TC	Li	FDA 71-85	5.0%	19	23	43	25	45	56
	Lu	FDA 71-85	5.0%	20	10	40	44	71	69
	T	FDA 71-85	5.0%	17	18	28	33	72	56

NOTE: TC = test compound
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2
(c) = contamination present

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VIII. NON-ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

DATE: 9-8-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
PC	TA-1535	EMS	0.05 %	2.96	1,790	604.73
	TA-1537	QM	0.01 mg/ml	3.11	911	292.93
	TA-1538	NF	1.25 mg/ml	3.66	266	72.68
SC	TA-1535	SALINE	-	3.27	6	1.83
	TA-1537	SALINE	-	4.66	37	7.94
	TA-1538	DMSO	-	3.01	13	4.32

NOTE: PC = positive control
SC = solvent control
EMS = ethyl methanesulfonate
QM = quinacrine mustard
NF = nitrosofluorene
DMSO = dimethyl sulfoxide
(c) = contamination present

NON-ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

DATE: 9-8-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ml x 10^8	his+ Revertants/ml	his+ Revertants/10^8 Survivors
TC	TA-1535	FDA 71-85	H	3.55 (109)	79	22.25
TC	TA-1535	FDA 71-85	L	4.52 (138)	44	9.74
TC	TA-1537	FDA 71-85	H	10.07 (216)	69	6.85
TC	TA-1537	FDA 71-85	L	6.76 (145)	71	10.50
TC	TA-1538	FDA 71-85	H	3.40 (113)	99	29.12
TC	TA-1538	FDA 71-85	L	4.78 (159)	72	15.06

NOTE: TC = test compound
H = high dose
L = low dose
(c) = contamination present
( ) = percent survival

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IX. ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MOUSE

DATE: 9-10-74				Strain TA-1535		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	DMNA	100 µmoles/ml	4.11	4,449	1082.48
	Lu	DMNA	100 µmoles/ml	2.59	24	9.27
	T	DMNA	100 µmoles/ml	4.14	22	5.31
SC	-	DMNA	100 µmoles/ml	3.37	6	1.78
	-	SALINE	-	3.84	8	2.08
DATE: 9-23-74				Strain TA-1537		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	4.64	80	17.24
	Lu	AAF	1.25 mg/ml	5.76	10	1.74
	T	AAF	1.25 mg/ml	5.04	22	4.37
SC	-	AAF	1.25 mg/ml	5.24	41	7.82
	-	DMSO	-	6.01	41	6.82
DATE: 9-12-74				Strain TA-1538		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	3.95	133	33.67
	Lu	AAF	1.25 mg/ml	4.47	53	11.86
	T	AAF	1.25 mg/ml	4.29	30	6.99
SC	-	AAF	1.25 mg/ml	4.66	10	2.15
	-	DMSO	-	4.84	33	6.82

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
DMSO = dimethyl sulfoxide

(c) = contamination present

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ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

SPECIES: Mouse

Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
DATE: 9-10-74				Strain TA-1535		
TC	Li	FDA 71-85	H	4.35 (113)	76	17.47
		FDA 71-85	L	3.60 (94)	94	26.11
	Lu	FDA 71-85	H	5.17 (135)	236 (c)	45.65
		FDA 71-85	L	5.35 (139)	124 (c)	23.18
	T	FDA 71-85	H	6.75 (176)	186	27.56
		FDA 71-85	L	5.57 (145)	93	16.70
DATE: 9-23-74				Strain TA-1537		
TC	Li	FDA 71-85	H	5.25 (87)	65	12.38
		FDA 71-85	L	4.12 (69)	60	14.56
	Lu	FDA 71-85	H	7.76 (129)	45	5.80
		FDA 71-85	L	6.31 (105)	76	12.04
	T	FDA 71-85	H	6.40 (106)	60 (c)	9.38
		FDA 71-85	L	5.67 (94)	75	13.23
DATE: 9-12-74				Strain TA-1538		
TC	Li	FDA 71-85	H	5.49 (113)	379	69.04
		FDA 71-85	L	3.78 (78)	547	144.71
	Lu	FDA 71-85	H	5.52 (114)	743	134.60
		FDA 71-85	L	4.72 (98)	878	186.02
	T	FDA 71-85	H	5.42 (112)	220	40.59
		FDA 71-85	L	5.90 (122)	148	25.09

NOTES: H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
( ) = percent survival

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ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: RAT

DATE: 9-13-74				Strain TA-1535		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	DMNA	100 µmoles/ml	3.83	1,342	350.39
	Lu	DMNA	100 µmoles/ml	4.34	9	2.07
	T	DMNA	100 µmoles/ml	3.39	15	4.43
SC	-	DMNA	100 µmoles/ml	3.14	5	1.59
	-	SALINE	-	4.13	7	1.70
DATE: 9-25-74				Strain TA-1537		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	4.93	146	29.62
	Lu	AAF	1.25 mg/ml	5.15	64	12.43
	T	AAF	1.25 mg/ml	4.87	79	16.22
SC	-	AAF	1.25 mg/ml	6.30	110	17.46
	-	DMSO	-	6.93	67	9.67
DATE: 9-24-74				Strain TA-1538		
Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
PC	Li	AAF	1.25 mg/ml	3.26	137	42.02
	Lu	AAF	1.25 mg/ml	3.18	33	10.38
	T	AAF	1.25 mg/ml	2.30	37	16.09
SC	-	AAF	1.25 mg/ml	1.46	24	16.44
	-	DMSO	-	2.85	37	12.98

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
DMSO = dimethyl sulfoxide

(c) = contamination present

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ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

SPECIES: Rat

Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
DATE: 9-13-74				Strain TA-1535		
TC	Li	FDA 71-85	H	3.81 (92)	99	25.98
		FDA 71-85	L	4.06 (98)	74	18.23
	Lu	FDA 71-85	H	3.91 (95)	290 (c)	74.17
		FDA 71-85	L	4.00 (97)	74 (c)	18.50
	T	FDA 71-85	H	4.05 (98)	205	50.62
		FDA 71-85	L	3.72 (90)	84	22.58
DATE: 9-25-74				Strain TA-1537		
TC	Li	FDA 71-85	H	5.92 (85)	131	22.13
		FDA 71-85	L	8.17 (118)	116	14.20
	Lu	FDA 71-85	H	5.75 (83)	110	19.13
		FDA 71-85	L	9.52 (137)	151	15.86
	T	FDA 71-85	H	7.42 (107)	114	15.36
		FDA 71-85	L	6.69 (97)	124	18.54
DATE: 9-24-74				Strain TA-1538		
TC	Li	FDA 71-85	H	4.73 (166)	199	42.07
		FDA 71-85	L	5.29 (186)	103	19.85
	Lu	FDA 71-85	H	5.41 (190)	149	27.54
		FDA 71-85	L	5.39 (189)	115	21.34
	T	FDA 71-85	H	6.07 (213)	- (c)	-
		FDA 71-85	L	4.29 (151)	112	26.11

NOTES:
H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
( ) = percent survival

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MONKEY

Test	Organ	Compound	Concentration	Total Cells/ mlx10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
DATE: 10-1-74				Strain TA-1535		
PC	Li	DMNA	100 µmoles/ml	5.33	1,600	300.19
	Lu	DMNA	100 µmoles/ml	5.91	10	1.69
	T	DMNA	100 µmoles/ml	5.91	5	0.85
SC	-	DMNA	100 µmoles/ml	4.24	9	2.12
	-	SALINE	-	5.24	9	1.72
DATE: 9-25-74				Strain TA-1537		
PC	Li	AAF	1.25 mg/ml	3.90	95	24.36
	Lu	AAF	1.25 mg/ml	2.95	22	7.46
	T	AAF	1.25 mg/ml	5.29	28	5.29
SC	-	AAF	1.25 mg/ml	3.18	25	7.86
	-	DMSO	-	2.66	15	5.64
DATE: 10-4-74				Strain TA-1538		
PC	Li	AAF	1.25 mg/ml	6.50	197	30.31
	Lu	AAF	1.25 mg/ml	6.87	86	12.52
	T	AAF	1.25 mg/ml	4.36	69	15.83
SC	-	AAF	1.25 mg/ml	4.90	84	17.14
	-	DMSO	-	5.55	71	12.79

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes
DMSO = dimethyl sulfoxide

(c) = contamination present

Project No. 2468

Litton BIONETICS

ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

SPECIES: Monkey

Test	Organ	Compound	Concentration	Total Cells/ ml x 10^8	his+ Revertants/ ml	his+ Revertants/10^8 Survivors
DATE: 10-1-74				Strain TA-1535		
TC	Li	FDA 71-85	H	6.53 (125)	14	2.14
		FDA 71-85	L	6.67 (127)	20	3.00
	Lu	FDA 71-85	H	7.34 (136)	- (c)	-
		FDA 71-85	L	5.64 (108)	22	3.90
	T	FDA 71-85	H	6.45 (123)	12	1.86
		FDA 71-85	L	5.64 (108)	13	2.31
DATE: 9-25-74				Strain TA-1537		
TC	Li	FDA 71-85	H	5.58 (210)	14	2.51
		FDA 71-85	L	4.10 (154)	59	14.39
	Lu	FDA 71-85	H	5.12 (197)	62	12.11
		FDA 71-85	L	5.76 (288)	75	13.02
	T	FDA 71-85	H	4.75 (179)	78	16.42
		FDA 71-85	L	5.58 (210)	43	7.71
DATE: 10-4-74				Strain TA-1538		
TC	Li	FDA 71-85	H	10.01 (180)	185	18.48
		FDA 71-85	L	7.26 (131)	153	21.07
	Lu	FDA 71-85	H	7.49 (135)	164	21.90
		FDA 71-85	L	7.78 (140)	178	22.88
	T	FDA 71-85	H	8.83 (159)	191	21.63
		FDA 71-85	L	7.67 (138)	144	18.77

NOTES: H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
( ) = percent survival

Project No. 2468

X. NON-ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 9-16-74

			Strain D4				
Test	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	EMS	1.0 %	6.94	189	271	27.23	39.05
SC	Saline	-	7.69	27	38	3.51	4.94

NOTE: PC = positive control
SC = solvent control
EMS = ethyl methanesulfonate

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

NON-ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 9-16-74

				Strain D4			
Test	Compound	Concentration	Total Population Screened[a]	Number Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	FDA 71-85	H	6.51 (85)	24	28	3.69	4.30
	FDA 71-85	L	6.30 (82)	32	17	5.08	2.70

NOTE: TC = test compound
H = high dose
L = low dose
a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present
( ) = percent survival

Project No. 2468

XI. ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Mouse

DATE: 9-19-74

					Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	Li	DMNA	150 µmoles/ml	5.16	82	77	15.89	14.92
	Lu	DMNA	150 µmoles/ml	4.93	13	10	2.64	2.03
	T	DMNA	150 µmoles/ml	5.10	11	6	2.16	1.18
SC	-	DMNA	150 µmoles/ml	6.51	20	26	3.07	3.99
	-	SALINE	-	6.47	37	22	5.72	3.40

NOTE: PC = positive control
SC = solvent and chemical controls
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: Mouse DATE: 9-19-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	Li	FDA 71-85	H	7.15 (111)	16	24	2.24	3.36
		FDA 71-85	L	6.34 (98)	29	28	4.57	4.42
	Lu	FDA 71-85	H	5.70 (88)	28	28	4.91	4.91
		FDA 71-85	L	5.54 (86)	26	16	4.69	2.89
	T	FDA 71-85	H	6.15 (95)	28	26	4.55	4.23
		FDA 71-85	L	7.33 (113)	14	17	1.91	2.32

NOTE:
TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10^5
b = number at 10^{-1} dilution
(c)= contamination present
( )= percent survival

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Rat DATE: 10-18-74

High Dose Strain D4

Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	Li	DMNA	150 µmoles/ml	7.93	78	75	9.84	9.46
	Lu	DMNA	150 µmoles/ml	7.99	33	27	4.13	3.37
	T	DMNA	150 µmoles/ml	9.03	36 (c)	31 (c)	3.99	3.43
SC	-	DMNA	150 µmoles/ml	8.74	42 (c)	31 (c)	4.81	3.55
	-	SALINE	-	7.13 (c)	43 (c)	24	6.03	3.37

NOTE: PC = positive control
SC = solvent and chemical controls
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: Rat DATE: 10-18-74

High Dose Strain D4

Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	Li	FDA 71-85	H	7.17 (101)	42	31	5.86	4.32
			L					
	Lu	FDA 71-85	H	7.70 (108)	47	40	6.10	5.19
			L					
	T	FDA 71-85	H	7.03 (99)	34	83	4.84	11.80
			L					

NOTE:
TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10^5
b = number at 10^{-1} dilution
(c)= contamination present
( )= percent survival

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Rat

DATE: 9-26-74

Low Dose

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
PC	Li	DMNA	150 µmoles/ml	5.85	73	70	12.48	11.97
	Lu	DMNA	150 µmoles/ml	5.81	31	11	5.34	1.89
	T	DMNA	150 µmoles/ml	5.00	32	19	6.40	3.80
SC	-	DMNA	150 µmoles/ml	5.27	31	23	5.88	4.36
	-	SALINE	-	5.50	28	20	5.09	3.64

NOTE: PC = positive control
SC = solvent and chemical controls
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: Rat DATE: 9-26-74

Low Dose Strain D4

Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	Li		H					
		FDA 71-85	L	5.27 (96)	32	16	6.07	3.04
	Lu		H					
		FDA 71-85	L	4.51 (82)	27	16	5.99	3.55
	T		H					
		FDA 71-85	L	6.19 (113)	17	9	2.75	1.45

NOTE:
TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10^5
b = number at 10^{-1} dilution
(c)= contamination present
( )= percent survival

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Monkey

DATE: 10-22-74

				Strain D4			
				Total Population Screened[a]	Number of Convertants[b]		Convertants Per 10^5 Survivors	
Test	Organ	Compound	Concentration		Ade^+	Try^+	Ade^+	Try^+
PC	Li	DMNA	150 µmoles/ml	5.88	68	67	11.56	11.39
	Lu	DMNA	150 µmoles/ml	5.26	30	28	5.70	5.32
	T	DMNA	150 µmoles/ml	5.85	31	40	5.30	6.83
SC	-	DMNA	150 µmoles/ml	4.89	27	44	5.52	9.00
	-	SALINE	-	4.14	34	35	7.95	8.21

NOTE: PC = positive control
SC = solvent and chemical controls
DMNA = dimethylnitrosamine
Li = liver
Lu = lung
T = testes

a = number x 10^5
b = number at 10^{-1} dilution
(c) = contamination present

Project No. 2468

ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: Monkey DATE:

					Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened[a]	Number of Convertants[b] Ade+	Try+	Convertants Per 10^5 Survivors Ade+	Try+
TC	Li	FDA 71-85	H	7.29 (176)	38 (c)	31	5.21	4.25
		FDA 71-85	L	7.10 (171)	40 (c)	70 (c)	5.63	9.86
	Lu	FDA 71-85	H	6.05 (146)	35	25	5.79	4.13
		FDA 71-85	L	6.96 (168)	32	49	4.60	7.04
	T	FDA 71-85	H	6.23 (150)	40 (c)	38 (c)	6.42	6.10
		FDA 71-85	L	(c) 6.98 (169)	22 (c)	53 (c)	3.15	7.59

NOTE:
TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10^5
b = number at 10^{-1} dilution
(c)= contamination present
( )= percent survival

Project No. 2468

XII. SUMMARY OF TEST RESULTS COMPOUND FDA 71-85

A. Suspension Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Reversion Frequencies (x 10^{-8}) TA-1535	TA-1537	TA-1538	Saccharomyces D4 Conversion Frequencies (x 10^{-5}) Ade^+	Try^+
NA-PC	-	-	604.73	292.93	72.68	27.23	39.05
NA-NC	-	-	1.83	7.94	4.32	3.51	4.94
NA-H	-	-	22.25	6.85	29.12	3.69	4.30
NA-L	-	-	9.74	10.50	15.06	5.08	2.70
A-NC (-C)	-	-	2.08	6.82	6.82	5.72	3.40
A-NC (+C)	-	-	1.78	7.82	2.15	3.07	3.99
A-PC	M	Li	1082.48	17.24	33.67	15.89	14.92
A-PC	M	Lu	9.27	1.74	11.86	2.64	2.03
A-PC	M	T	5.31	4.37	6.99	2.16	1.18
A-H	M	Li	17.47	12.38	69.04	2.24	3.36
A-L			26.11	14.56	144.71	4.57	4.42
A-H	M	Lu	45.65	5.80	134.60	4.91	4.91
A-L			23.18	12.04	186.02	4.69	2.89
A-H	M	T	27.56	9.38	40.59	4.55	4.23
A-L			16.70	13.23	25.09	1.91	2.32

a NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

b M = mouse
Mo = monkey
R = rat

c Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

COMPOUND FDA 71-85

B. Plate Tests

Test[a]	Activation: Species[b]	Activation: Organ[c]	Salmonella Responses: TA-1535	TA-1537	TA-1538
NA-PC	-	-	+	+	+
NA-NC	-	-	-	-	-
NA-H	-	-	-	-	-
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	M	Li	+	+	+
A-PC	M	Lu	-	-	-
A-PC	M	T	-	-	-
A-H	M	Li	-	-	-
A-H	M	Lu	-	-	-
A-H	M	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

SUMMARY OF TEST RESULTS COMPOUND FDA 71-85

A. Suspension Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Reversion Frequencies (x 10^{-8}) TA-1535	TA-1537	TA-1538	Saccharomyces D4 Conversion Frequencies (x 10^{-5}) Ade+ L	Ade+ H	Try+ L	Try+ H
NA-PC	-	-							
NA-NC	-	-							
NA-H	-	-							
NA-L	-	-							
A-NC (-C)	-	-	1.70	9.67	12.98	5.09	6.03	3.64	3.37
A-NC (+C)	-	-	1.59	17.46	16.44	5.88	4.81	4.36	3.55
A-PC	R	Li	350.39	29.62	42.02	12.48	9.84	11.97	9.46
A-PC	R	Lu	2.07	12.43	10.38	5.34	4.13	1.89	3.37
A-PC	R	T	4.43	16.22	16.09	6.40	3.99	3.80	3.43
A-H	R	Li	25.98	22.13	42.07	5.86		4.32	
A-L	R		18.23	14.20	19.85	6.07		3.04	
A-H	R	Lu	74.17	19.13	27.54	6.10		5.19	
A-L	R		18.50	15.86	21.34	5.99		3.55	
A-H	R	T	50.62	15.36	-	4.84		11.80	
A-L	R		22.58	18.54	26.11	2.75		1.45	

a NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

b M = mouse
Mo = monkey
R = rat

c Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

COMPOUND FDA 71-85

B. Plate Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Responses TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	R	Li	+	+	+
A-PC	R	Lu	-	-	-
A-PC	R	T	-	-	-
A-H	R	Li	-	-	-
A-H	R	Lu	-	-	-
A-H	R	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

SUMMARY OF TEST RESULTS COMPOUND FDA 71-85

A. Suspension Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Reversion Frequencies (x 10^{-8}) TA-1535	TA-1537	TA-1538	Saccharomyces D4 Conversion Frequencies (x 10^{-5}) Ade^+	Try^+
NA-PC	-	-					
NA-NC	-	-					
NA-H	-	-					
NA-L	-	-					
A-NC (-C)	-	-	1.72	5.64	12.79	8.45	8.21
A-NC (+C)	-	-	2.12	7.86	17.14	5.52	9.00
A-PC	Mo	Li	300.19	24.36	30.31	11.56	11.39
A-PC	Mo	Lu	1.69	7.46	12.52	5.70	5.32
A-PC	Mo	T	0.85	5.29	15.83	5.30	6.84
A-H	Mo	Li	2.14	2.51	18.48	5.21	4.25
A-L	Mo		3.00	14.39	21.07	5.63	9.86
A-H	Mo	Lu	-	12.11	21.90	5.79	4.13
A-L	Mo		3.90	13.02	22.88	4.60	7.04
A-H	Mo	T	1.86	16.42	21.63	6.42	6.10
A-L	Mo		2.31	7.71	18.77	3.15	7.59

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

COMPOUND 71-85

B. Plate Tests

Test[a]	Activation: Species[b]	Activation: Organ[c]	Salmonella Responses: TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	Mo	Li	+	+	+
A-PC	Mo	Lu	-	-	-
A-PC	Mo	T	-	-	-
A-H	Mo	Li	-	-	-
A-H	Mo	Lu	-	-	-
A-H	Mo	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

Project 2468

XIII. INTERPRETATION AND CONCLUSIONS

Compound FDA 71-85, Hydrolyzed Vegetable Protein (Soy), was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained.

A. Comments

In most tests with all three *Salmonella* his^- mutants the reversion frequencies were elevated several times over the spontaneous frequencies (saline negative control). This elevation occurred in plate assays as well as suspension tests. The results appear to be the consequence of histidine residues in the hydrolyzed vegetable protein which produce both an increased spontaneous background and phenotypic revertants which are not in fact his^+ colonies. This phenomenon can be reduced somewhat by scoring minimal plates earlier than usual, as can be seen in the activation suspension tests with monkey tissues. High background makes evaluation of this compound difficult but the results when viewed in the light of compound enrichment do not suggest any genetic activity for FDA 71-85.

B. *Salmonella typhimurium*

1. Plate Tests

At a concentration of 5.0%, this chemical was not mutagenic in direct or activation plate tests with strains TA-1535, TA-1537 or TA-1538.

2. Non-activation Suspension Tests

These tests were all negative.

3. Activation Suspension Tests

These tests were all negative.

C. *Saccharomyces cerevisiae*

1. Non-activation Suspension Tests

These tests were all negative.

D. Conclusions

This compound was not genetically active under the test conditions employed in this evaluation.

David Brusick, Ph.D.
Director
Department of Genetics

APPENDIX

SUMMARY OF TESTS EVALUATING DMSO FOR GENETIC ACTIVITY IN *SALMONELLA* AND *SACCHAROMYCES*

Litton BIONETICS

COMPOUND DIMETHYSULFOXIDE

A. Suspension Tests

Test	Activation Species[a]	Activation Organ[b]	Salmonella Reversion Frequencies ($\times 10^{-8}$) TA-1535	Salmonella Reversion Frequencies ($\times 10^{-8}$) TA-1538	Saccharomyces D4 Conversion Frequencies ($\times 10^{-5}$) Ade^+	Saccharomyces D4 Conversion Frequencies ($\times 10^{-5}$) Try^+
Non-activation						
Control (-C)	-	-	0.74	0.88	32.51	4.34
High Dose[c]	-	-	1.91	1.05	28.32	2.95
Low Dose[d]	-	-	0.53	1.37	40.73	0.49
Activation						
Control (+C)	-	-	1.80	0.36	38.27	2.47
Control (-C)	-	-	1.43	1.04	37.12	2.64
High Dose[c]	M	Li	0.34	1.07	47.77	2.75
	M	Lu	0.59	0.58	36.29	1.39
	M	T	0.62	0.30	34.35	1.94
Lose Dose[d]	M	Li	-	0.87	34.02	1.18
	M	Lu	0.43	3.14	42.30	1.40
	M	T	0.11	0.39	45.95	2.32

Note: (-C) = solvent control and (+C) = test chemical control without homogenate

a M = mouse, Mo = monkey, R = rat

b Li = liver, Lu = lung, T = testes

c Bacteria = 3%, Yeast = 5%

d Bacteria = 1.5%, Yeast = 2.5%

COMPOUND DIMETHYSULFOXIDE

B. Plate Tests

Test	Activation: Species[a]	Activation: Organ[b]	Salmonella Responses: TA-1535	TA-1537	TA-1538
Non-activation					
Control (-C)	-	-	-	-	-
Test compound (3%)	-	-	-	-	-
Activation					
Control (+C)	-	-	-	-	-
Control (-C)	-	-	-	-	-
Test compound (3%)	M	Li	-	-	-
	M	Lu	-	-	-
	M	T	-	-	-
	R	Li	-	-	-
	R	Lu	-	-	-
	R	T	-	-	-
	Mo	Li	-	-	-
	Mo	Lu	-	-	-
	Mo	T	-	-	-

Note: (-C) = solvent control and (+C) = chemical control without homogenate

a M = mouse
Mo = monkey
R = rat

b Li = liver
Lu = lung
T = testes